

Variation in phenol, electrical conductivity, and sugar contents in *Grewia oppositifolia* Roxb.

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Abstract: We estimated variation in the allocation of metabolites (phenol, sugar, and electrical conductance) in seed, fruit and seedling leachates of 25 populations of *Grewia oppositifolia*. Higher quantity of phenolics and higher electrical conductance were observed in plants from lower-middle altitudes but this was not correlated significantly with germination, survival, or plant height. On the other hand, sugar content in fruit pulp leachates was greatest in higher elevational populations and these sources also recorded higher germination, survival, and plant height under nursery conditions. Thus we recommend that germplasm (seed) should be collected from moderately higher elevational sources for nursery as well as future breeding strategies of this potential agroforestry tree crop of the central Himalaya.

Keywords: *Grewia oppositifolia*; phenol; electrical conductivity; sugar; seed; seedling; Garhwal Himalaya

Introduction

The geographical areas and environments in which parent trees grow, and within which their genetic constitution has been developed through natural and artificial selection, result in variation among natural populations of a species (Uniyal et al. 2000 & 2003). Basic knowledge about nature and extent of seed source variation with respect to important primary metabolites like phenols, sugar exudates, and electrical conductivity which are directly linked with vigour and germination is essential for future genetic improvement of the species, especially for early selection (Ramchandra 1996).

Extensive work has been undertaken on assessment of nutri-

tive value (Khosla et al. 1992) and socio-economic importance (Todaria and Bhatt 1992; Negi et al. 1999) of *Grewia oppositifolia*, but no work has been carried out on the variation in metabolites and their influence on overall performance of this species. Though, certain metabolites such as phenols, sugar exudates, and conductivity of leachates are directly responsible for seed vigour and accelerated ageing (Singh 2004).

G. oppositifolia Roxb. (Family - Tiliaceae) is a fodder tree growing in the Himalaya from Jammu & Kashmir to Nepal up to elevations of about 2,000 m.a.s.l. (Troup 1921). It has been grown in Garhwal Himalaya for farming under agroforestry practices due to its multipurpose nature. The extent of tree-to-tree variation in number of biochemicals in fruit and seed traits of some indigenous fruit tree species has been studied in *Irvingia gabonensis* (Leakey et al. 2000; Atangana et al. 2002), *Canarium schweinfurthii* (Kapchie et al. 2002) and *Dacryodes edulis* (Leakey et al. 2003). Variation among biochemicals in seed traits in *G. oppositifolia*, however, has not been well documented. The establishment of correlation between germination traits and biochemical traits would contribute not only to a better understanding of the phenomenon of seed dormancy in these species, but also to an efficient orientation of research aimed at improving seed germination. In view of these issues, we undertook this study to evaluate the allocation and possible influence of these important metabolites in seed, seedling, and fruit leachates of *G. oppositifolia* in the context of source variation for early screening of desired sources to conduct future improvement programmes in this potential agroforestry tree species of the central Himalaya.

Materials and methods

This study was carried out to evaluate variation in phenols, sugar exudates and conductivity of various leachates in relation to various seed and seedling parameters of *G. oppositifolia*. Twenty five seed sources were selected for this study on the basis of altitude, longitudinal, and latitudinal variations in natural habitat of this species (Table 1). For each source, fresh mature (ripe fruit

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black in colour) seeds were harvested from 25 pre selected ideotypes, which were 100 to 300 meters apart to avoid narrowing down the variation in samples due to relatedness or inbreeding (ISTA 1993). Seeds from the parent trees were randomly mixed to make a composite sample for each source separately.

Table 1. Geographical and meteorological description of seed sources of *G. oppositifolia*

Provenance	Altitude (m.a.s.l.)	Latitude (N)	Longitude (E)	Temperature (°C)	Rainfall (mm)
1 Kotdwara	366	29°47'	78°32'	22.10	180.29
2 Bilkhet	450	29°57'	78°43'	26.30	168.21
3 Karanpryag	457	30°32'	78°05'	24.50	235.75
4 Fatehpur	510	30°12'	78°10'	29.40	159.00
5 Srinagar	560	30°13'	78°48'	23.70	157.00
6 Bilashpur	675	31°35'	76°58'	28.82	089.30
7 Tehri	700	30°25'	78°28'	27.63	080.00
8 Simswara	850	30°06'	78°35'	25.08	087.22
9 Augastyamuni	875	30°21'7"	78°59'	29.53	083.30
10 Ganai	890	30°27'	78°39'	28.60	123.00
11 Nahan	915	30°34'	78°18'	28.10	092.60
12 Chham	925	30°31'	78°22'	28.06	123.00
13 Malshi	960	30°26'	78°05'	28.02	159.92
14 Dov	1015	30°10'	78°47'8"	20.30	070.00
15 Ghansali	1100	29°52'	79°22'	24.00	150.30
16 Shyalde	1200	29°47'	79°15'	25.50	105.70
17 Naogaon	1300	30°48'	79°09'	27.61	149.86
18 Nauni	1310	30°15'	77°08'	25.08	087.22
19 Guptakashi	1400	30°16'	79°15'	24.10	125.75
20 Mayali	1400	30°23'7"	78°47'	24.10	103.00
21 Chilledi	1550	30°19'	78°49'	19.45	153.00
22 Niglat	1650	29°23'	79°31'	24.14	144.40
23 Solan	1650	30°57'	77°05'	27.70	070.50
24 Thati	1700	30°17'	78°50'	19.45	120.00
25 Syunsal	1750	29°48'	79°12'	26.15	140.00

Rainfall is recorded on average monthly basis.

Biochemical estimation

Total phenolic contents of whole seed, fruit pulp, and whole seedling (after three months growth) were estimated by the method described by Bray and Thorpe (1954). Total soluble sugar was determined by the method prescribed by Dubois et al. (1951). Electrical conductivity (EC) of seed leachates was measured by using a digital conductivity meter (model – AP X 185) by immersing the rod into the leachates.

Laboratory germination

For seed germination study, five replicates (each of 100 seeds) of each population were soaked in hot water for 24 h (Uniyal et al. 2000) and then placed on petri dishes (9 cm diameter) containing

Whatman No.-1 filter paper moistened with distilled water. Replicates were arranged in randomized block design (RBD) in a seed germinator fixed at 25°C in dark conditions as maximum germination was recorded at this temperature in earlier studies (Uniyal et al. 2000). Filter paper was moistened daily using distilled water. Germination was recorded when the radical emerged and the test was completed after 28 days. Observation was made daily by counting the number of germinated seeds.

Nursery germination and survival

Seeds from all 25 sources were presoaked in hot water for 24 h thereafter sown at 10 cm spacing in nursery beds of 1 × 1 m size in RBD fashion with four replicates (each of 100 seeds) in experimental Garden-Chauras, Srinagar Garhwal (situated between 30°13' N and 78°48' E at 569 m.a.s.l.). Watering was done regularly until the completion of germination and thereafter, three days each week until the onset of the rainy season. Weeding was done manually. Seed germination in the nursery was recorded only after the start of plumule extension above the soil. Survival percent of this experiment was recorded after completion of one year of seed germination. The results were statistically analyzed by using mean values, ANOVA, Tukey –test (Bartz 1988), simple correlation coefficient (Sharma 1998).

Tukey test:

$$Q \geq \frac{\overline{X}_L - \overline{X}_S}{\sqrt{\frac{MS_{WGz}}{N_G}}}$$

where \overline{X}_L is the larger of the two mean, \overline{X}_S is the smaller of the two means, MS_{WG} is the number of replications, $q = q(\alpha; k; DF)$ with α = significance level, k = number of populations (of means) and DF = df of the error form in ANOVA.

Results and discussion

Estimation of phenol contents in seed, fruit pulp, and seedling leachate revealed considerable variation among the sources. Maximum phenol content in seed leachate (0.34 mg/gm) was observed in Ganai and Mayali populations, these were closely followed by Kotdwara and Dov (both 0.33 mg/gm), while the minimum value (0.14 mg/gm) was recorded in Niglat provenance. Phenol contents in fruit pulp leachate varied from 0.15 to 0.35 mg/gm, respectively in Mayali and Tehri sources, while Srinagar, Ghansali, Nahan (all 0.34 mg/gm) and Naogaon & Thati (0.16 & 0.17 mg/gm) sources were significantly at par with Tehri and Mayali populations, respectively. Phenol contents in seedling leachates were greatest (0.34 mg/gm) in Fatehpur and Srinagar sources, while the minimum value (0.15 mg/gm) was recorded in Syunsal population (Table 2).

Table 2. Variation in phenol, sugar, electrical conductivity in different leachates and laboratory/nursery germination, survival percent & plant height of various sources of *G. oppositifolia*

Provenance	Phenol			Sugar Fruit	EC seed	Germination (%)		Survival %	Plant height
	Seed	Fruit	Seedling			Lab	Nursery		
1 Kotdwara	0.33 ^a	0.30 ^{de}	0.33 ^{ab}	8.55 ^{bc}	7.20 ^{abc}	55.50 ^{bc}	33.33 ^b	25.00 ^j	102.20 ^{bc}
2 Bilkhet	0.24 ^{hi}	0.25 ^{gh}	0.32 ^{bc}	6.11 ^h	2.80 ^{lm}	31.50 ^{kl}	28.67 ^{def}	43.82 ^{hi}	95.40 ^{de}
3 Karanpryag	0.32 ^{bc}	0.23 ^{ij}	0.31 ^{cd}	6.20 ^{gh}	6.80 ^{bcd}	56.00 ^{bcd}	33.00 ^{bc}	68.75 ^{de}	85.70 ^{hi}
4 Fatehpur	0.24 ^{hi}	0.28 ^d	0.34 ^a	6.68 ^{fg}	4.10 ^{ij}	39.00 ^h	24.00 ^{hi}	25.00 ^j	99.50 ^c
5 Srinagar	0.32 ^{bc}	0.34 ^a	0.34 ^a	8.95 ^b	6.20 ^{ef}	37.50 ^{hij}	19.67 ^{jk}	50.85 ^{gh}	102.50 ^{bc}
6 Bilashpur	0.21 ^j	0.32 ^{bc}	0.33 ^{ab}	6.11 ^h	3.80 ^{jk}	35.00 ^{ijk}	20.33 ^{jk}	39.34 ⁱ	80.00 ^j
7 Tehri	0.31 ^{cd}	0.35 ^a	0.32 ^{bc}	6.85 ^f	7.35 ^{ab}	40.00 ^{gh}	26.33 ^{fh}	52.00 ^{gh}	98.10 ^{cde}
8 Simswara	0.29 ^{ef}	0.30 ^{de}	0.30 ^d	6.92 ^f	6.60 ^{cde}	59.00 ^b	27.33 ^{efg}	54.88 ^{gh}	90.70 ^{fg}
9 Augastyamuni	0.30 ^{de}	0.30 ^{de}	0.24 ^g	8.89 ^{bc}	6.40 ^{de}	43.00 ^{fg}	29.33 ^{de}	56.82 ^{fg}	86.40 ^{hi}
10 Ganai	0.34 ^a	0.34 ^a	0.30 ^d	5.13 ⁱ	7.50 ^a	31.00 ^l	19.00 ^{kl}	36.86 ⁱ	84.28 ⁱ
11 Nahan	0.28 ^f	0.34 ^a	0.32 ^{bc}	5.20 ⁱ	7.50 ^a	38.00 ^{hi}	16.67 ^l	36.00 ⁱ	112.20 ^a
12 Chham	0.31 ^{cd}	0.29 ^{ef}	0.30 ^d	8.41 ^{bc}	4.70 ^{hi}	48.00 ^e	30.67 ^{cd}	52.17 ^{gh}	98.70 ^{cd}
13 Malshi	0.30 ^{de}	0.26 ^g	0.31 ^{cd}	7.83 ^{de}	2.50 ^m	37.00 ^{hij}	21.00 ^{jk}	78.95 ^{abc}	89.40 ^{gh}
14 Dov	0.33 ^{ab}	0.31 ^{cd}	0.26 ^f	7.79 ^{de}	4.30 ^{ij}	55.00 ^e	30.00 ^d	75.00 ^{cd}	102.00 ^{bc}
15 Ghansali	0.16 ^l	0.23 ^{ij}	0.24 ^g	6.75 ^{fg}	5.30 ^{gh}	45.00 ^{ef}	28.33 ^{def}	65.75 ^e	85.40 ^{hi}
16 Shyalde	0.26 ^g	0.30 ^{de}	0.18 ^{ij}	8.72 ^{bc}	5.70 ^{fg}	34.00 ^{kl}	20.00 ^{jk}	27.27 ^j	104.00 ^b
17 Naogaon	0.23 ⁱ	0.16 ^{lm}	0.27 ^{ef}	6.22 ^{gh}	3.20 ^{ke}	46.60 ^{ef}	28.33 ^{def}	87.27 ^a	91.00 ^{fg}
18 Nauni	0.31 ^{cd}	0.22 ^j	0.17 ^{jk}	8.39 ^{cd}	4.70 ^{hi}	45.00 ^{ef}	25.33 ^{gh}	86.44 ^{ab}	98.50 ^{cd}
19 Guptakashi	0.24 ^{hi}	0.32 ^{bc}	0.27 ^{ef}	5.80 ^h	5.00 ^h	57.00 ^{bc}	29.00 ^{de}	80.00 ^{abc}	113.40 ^a
20 Mayali	0.34 ^a	0.15 ^m	0.20 ^h	8.81 ^{bc}	6.90 ^{abcd}	63.00 ^a	28.55 ^{def}	78.26 ^{bc}	111.00 ^a
21 Chilledi	0.19 ^k	0.20 ^k	0.27 ^{ef}	6.20 ^{gh}	3.10 ^{lm}	33.00 ^{kl}	37.67 ^a	85.26 ^{ab}	95.30 ^{de}
22 Niglat	0.14 ^m	0.20 ^k	0.19 ^{hi}	8.79 ^{bc}	6.80 ^{bcd}	52.50 ^d	34.33 ^b	80.59 ^{abc}	115.00 ^a
23 Solan	0.18 ^k	0.24 ^{hi}	0.16 ^{kl}	5.73 ^{hi}	3.10 ^{lm}	44.00 ^f	22.00 ^{ijk}	61.72 ^{ef}	90.60 ^{fg}
24 Thati	0.25 ^{gh}	0.17 ^l	0.26 ^f	8.87 ^{bc}	5.00 ^h	44.00 ^f	39.00 ^a	82.16 ^{abc}	94.10 ^{ef}
25 Syunsal	0.19 ^k	0.22 ^j	0.15 ^l	9.95 ^a	2.60 ^{lm}	56.00 ^{bcd}	39.33 ^a	79.69 ^{abc}	114.20 ^a
Mean	0.26	0.26	0.27	7.35	5.17	45.02	27.65	60.39	97.58

Means followed by same later within each column are not significantly different at $p=0.05$

No relationship was found between the phenol content in all leachates and germination, survival and plant height (Table 3a). However, phenol content in seed was positively significantly ($p=0.05$) correlated with phenol content in fruit pulp, seedling leachates, and EC of seed leachates. Phenol content of fruit was significantly ($p=0.01$) but negatively correlated with germination as well survival percent in nursery. On the other hand, sugar content in fruit pulp was positively significantly ($p=0.05$) correlated with germination and plant height. Knowledge of the nature and extent of seed source variation with respect to important primary metabolites like proteins, sugars, starch, and free amino acids, which are directly linked to vigor and germination (Uniyal 1999; Saklani 1999; Singh 2004), is essential for genetic improvement of a species, especially for early selection (Ramchandra 1996; Rawat 2007) and socio-economic importance (Luna 2005), but no or little work has been carried out on variation of metabolites and their influence on overall performance of this species. These results revealed that phenol contents negatively affect the germination and survival in nursery. Similar variations have been found in seeds of many other tropical species such as *Dalbergia sissoo* (Khurana and Singh 2001), *Irvingia gabonensis* (Leakey et al. 2000; Atangana et al. 2002) *Ca-*

narium schweinfurthii (Kapchie et al. 2002) and *Dacryodes edulis* (Leakey et al. 2003). Similar studies have also been carried out with *Grewia optiva* (Uniyal 1999) and many other plant species, including *Quercus leucotrichophora* (Saklani 1999), *Celtis australis* (Singh 2004), *Abies pindrow*, *Cupressus torulosa* and *Picea smithiana* (Rawat 2007), *Balanites aegyptiaca* (Azo et al. 2005), and *Cicer arietinum* (Emenky and Khalaf 2008), for which correlations between seed size, biochemical attributes, and germination have been reported. In various other plant species, the earlier reports also attributed wide variation in germination traits to differences in allocation of some metabolites in various seed lots (Bewley and Black 1994; Malik and Shamet 2009). This study suggests that higher quantity of phenol content in seed coats and fruit pulp may inhibit germination and survival because phenols in seed coats and fruit pulp have been considered toxic to germination if present at higher concentrations (Ramchandra 1996). Highly significant and negative correlations have been observed between altitude and phenolics in seed, fruit pulp, and seedling leachates, indicating the reduction of phenolics with increasing altitude, which also signifies increase of phenolics with increasing air temperatures (phenolics in fruit content have positive and significant correlation at $p=0.05$ level with tempera-

ture), which is always higher at lower elevations (Table 3a & b). Similar correlations between metabolites and germination/ sur-

vival were reported for *Garcinia kola* heckel (Kanmegne et al. 2010).

Table 3a. Correlation coefficient among and between various leachates, germination, survival & plant height in *G. oppositifolia*

Variable	Phenol Seed	Phenol Fruit	Phenol Seedling	Sugar Fruit	EC Seedling	Laboratory G%	Nursery G%	Nursery Sur. %
Phenol Fruit	0.385*	-						
Phenol Seedling	0.377*	0.506**	-					
Sugar Fruit	0.124	-0.244	-0.409*	-				
EC Seedling	0.508**	0.215	0.132	0.070	-			
Laboratory G%	0.117	-0.295	-0.295	0.340*	0.264	-		
Nursery G%	-0.258	-0.569**	-0.257	0.402*	-0.128	0.494**	-	
Nursery Survival %	-0.235	-0.671**	-0.476**	0.202	-0.169	0.399*	0.525**	-
Plant Height	-0.058	-0.048	-0.310	0.381*	0.197	0.388*	0.198	0.121

Significant at $p=0.05$, ** Significant at $p=0.01$

Table 3 b. Correlation coefficient between geographical variables and various leachates, germination, survival & plant height in *G. oppositifolia*

Variable	Phenol Seed	Phenol Fruit	Phenol Seedling	Sugar Fruit	EC Seedling	Laboratory G%	Nursery G%	Nursery Sur. %	Plant Height
Altitude	-0.516**	-0.588**	-0.797**	0.233	-0.190	0.223	0.378*	0.685**	0.344*
Latitude	0.198	0.160	0.317	-0.451*	-0.164	-0.201	-0.352*	0.015	-0.364*
Longitude	-0.186	-0.169	-0.167	0.271	0.276	0.237	0.310	0.191	0.464**
Temperature	0.038	0.342*	0.137	-0.270	-0.030	-0.320	-0.438*	-0.417	-0.090
Rainfall	-0.065	-0.262	0.339*	-0.022	-0.001	0.025	0.410*	0.010	0.051

* Significant at $P=0.05$, ** Significant at $P=0.01$

Data for variation in sugar contents in fruit pulp leachates of 25 provenances are presented in Table 2. There were significant differences between minimum and maximum values for sugar content among the provenances. Maximum value (9.95 mol/gm fresh weight) was recorded at Syunsal, while minimum sugar content (5.13 mol/gm fresh weight) was observed at Ganai. In this case, sugar content in fruit pulp seems to enhance germination and increase plant height. Malik and Shamet (2009) also proposed the determination of various metabolites as a direct and rapid vigour test for seed viability. Sugar contents in fruit leachates have non significant relationships with all geographical variables, except for negative and significant ($p=0.05$) correlation with latitude (Table 3b). Berberis fruits from lower elevations, contained more sugar and a lower concentration of phenolics (Chandra and Todaria 1983; Todaria 1986). However, in this study maximum sugar content in fruit pulp was recorded in most of the higher elevational sources. This supports the earlier findings of Rawat and Uniyal (2011) in three conifer species of Garhwal Himalya, Saklani (1999) in *Quercus leucotrichophora*, and Singh (2004) in *Celtis australis*. Although, higher concentration of phenolics inhibit germination, once the water is imbibed by the seed coat, the concentration of phenol in the seed coat and fruit pulp is diluted which helps in initiation of germination. In *Grewia oppositifolia*, however, this takes a longer time because of the hard seed coat.

Significant variations were recorded for EC in seed leachates of 25 provenances (Table 2). The minimum EC value (2.5 $\mu\text{mhos}/\text{Cm}^2$) was recorded in seed leachates of Malsi while max-

imum EC (7.5 $\mu\text{mhos}/\text{Cm}^2$) was observed in Ganai and Nahan sources. EC of seed leachates has no relation with germination, survival, or plant height, however, the measurement of EC of leachates is universally accepted as related to seed quality and has been used as a technique to predict germination or quality of seeds of many forest tree species (Ramchandra 1996). On an average, sources having higher EC values have lower germination except for few provenances like Niglat, Mayali and Augast-yamuni. This concept is based on the fact that, when seeds are soaked in water, low vigour seeds release more electrolytes in the solution than high vigour seeds. EC showed non significant relationships with all geographical variables (Table 3b). Ramchandra (1996) reported similar findings on germination and average EC of sissoo seed.

Conclusion

Determination of important metabolites like phenols, sugar, and EC in seed, fruit pulp, and seedling leachates revealed significant differences among the seed lots of this species, which signify their role in germination, survival and plant height. Wide variation in germination and growth traits might be attributed to differences in some metabolite contents of various seed lots. However, these showed non significant correlations with all geographical variables. I conclude that most of the middle- higher elevation populations had lower concentrations of phenol and EC as well as higher sugar concentrations. These populations also

had higher germination, survival, and plant growth. Thus exploiting natural variation and using appropriate seed accessions as planting material could be promising tools for large-scale multiplication of this potential agroforestry tree species.

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